Supplementary file 1: Detailed methods

Detailed description of selection of quantotypic peptides for QconCAT construction

Proteotypic peptides for the 20 target proteins were selected from the Bovine PeptideAtlas (www.peptideatlas.org) based on their suitability score which takes into account both observations from our previous proteomics experiments and predicted observability derived from physiochemical properties.[19] Only unique and true tryptic peptides with a length of 7-20 amino acids which mapped to single genome locations were chosen. To avoid artefactual modifications during expression or synthesis, amino acids which are prone to oxidation (cysteine, methionine or tryptophan) were avoided in the target peptides whenever possible. Furthermore, peptides with potential post-translational modification sites such as serine and N-terminal glutamine were avoided where feasible. For the QconCAT construct, acidic residues in P1' (the amino acid position following the tryptic lysine/arginine cleavage site) were avoided as these residues can reduce the rate of trypsin cleavage.[20] In addition, missed or variable tryptic cleavages can occur due to neighboring basic amino acids at the tryptic cleavage site in a protein sequence and as such, peptides that generated dibasic sites were avoided.

Detailed description of QconCAT construction

The experimental methods for the expression of QconCAT proteins have previously been described in (Pratt et al. 2006). In addition to the 40 proteotypic peptides, a [Glu1]-FibrinopeptideB (Glufib) peptide and a His-tag was included in the QconCAT. The QconCAT DNA construct was optimized and synthesized *de novo* and cloned into pET21a by PolyQuant GmbH (Regensburg, Germany). *E.coli* strain BL21(λ)DE3 (*E.coli* B F^- *dcm ompT hsd*S_B(r_B^- m_B^-) *gal*, λ (DE3) was transformed with the vector and cultured in minimal medium (1xM9 salts (9), 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.00005% (w/v) thiamine, 0.2% (w/v) glucose, unlabelled amino acids at 0.1 mg/ml or 0.2 mg/ml

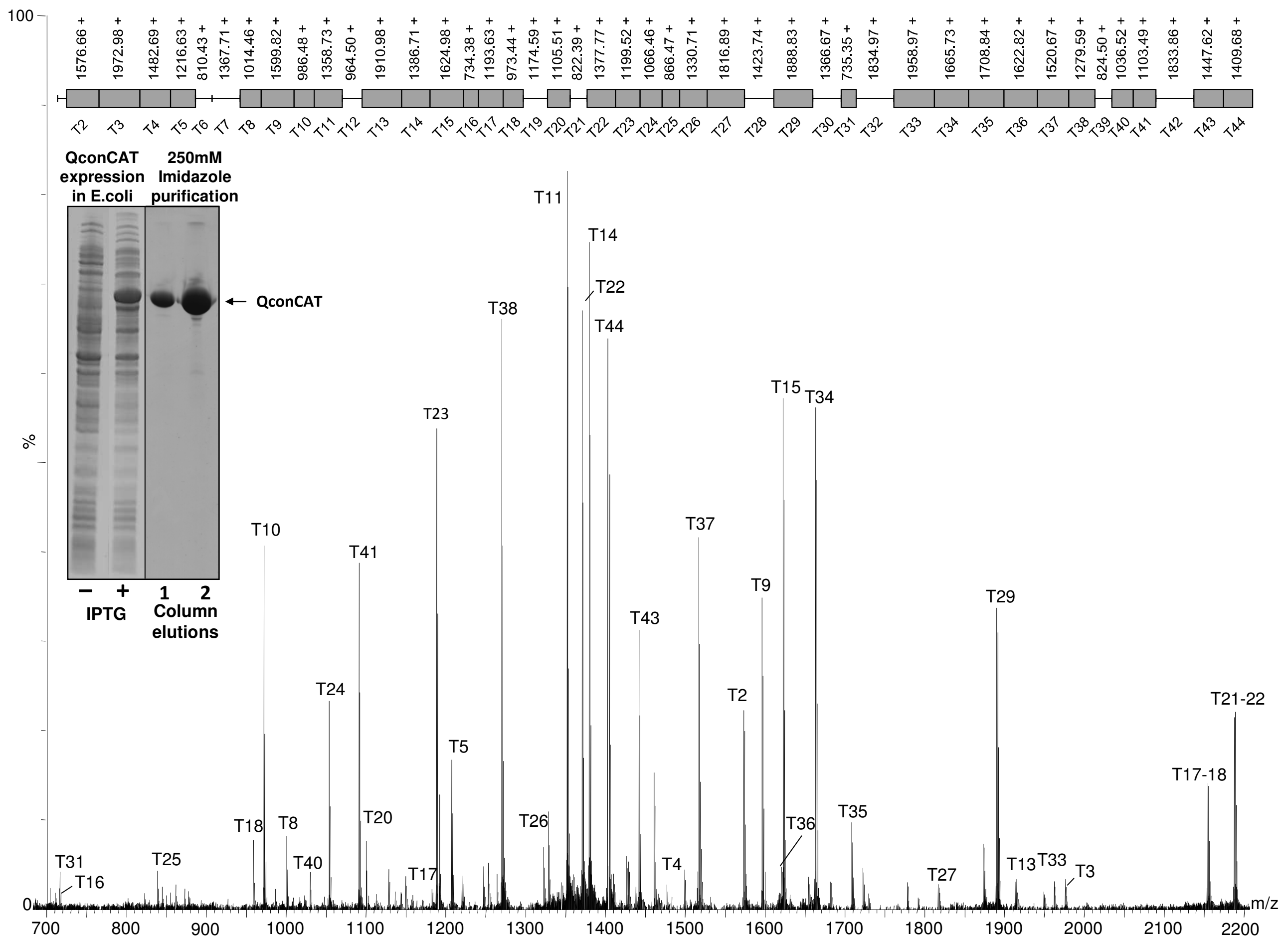
His, Tyr, Phe, Pro and Trp), supplemented either with unlabelled or [$^{13}C_6$]arginine and [$^{13}C_6$]lysine at 0.1 mg/ml. Cells were grown to mid log phase (OD_{600}=0.6-0.8) at which point expression was induced by adding 1 mM IPTG (isopropyl β-D-1-thiogalactopyranoside). Cells were lysed with the BugBuster Protein Extraction Reagent (Merck Chemicals, Nottingham, UK). After induction, the QconCAT is the predominant protein in the cell lysate and is present in inclusion bodies. Inclusion bodies were recovered by low speed centrifugation, redissolved in chaotropes and the recombinant QconCATs in labeled and unlabelled form were purified by anti-HisTag affinity chromatography to a high level of purity (for a detailed protocol see Pratt et al. 2006). MALDI-TOF mass spectrometry of the purified QconCAT confirmed that the protein was intact and that most of the tryptic peptides were readily discernible (Figure 1). The concentration of purified QconCAT protein was determined using the Pierce BCA Protein Kit (Bie and Berntsen, Denmark) with bovine serum albumin (BSA) as standard, according to manufacturer's manual. Four fmol of purified QconCAT protein was added to 1µg of every protein sample before tryptic digestion, hence 1 µg protein digest spiked with four fmol QconCAT peptides was injected on every SRM analysis. This QconCAT plasmid is available free of charge for research applications, and is distributed upon request from DNASU http://dnasu.asu.edu/DNASU/Home.jsp

Selection of optimal transitions

5-6 peptides from each protein, corresponding to native (light isotope) versions of the 40 QconCAT peptides, were synthesized using inexpensive Spot-synthesis technology (JPT Peptide Technologies GmbH, Berlin, Germany) and used without purification for optimizing the SRM assay conditions of the naturally occurring peptide analogues. Between 50 and 60 nmol of synthesized crude peptides were resuspended in 20% (v/v) ACN, 1% (v/v) formic acid, shaken for 60 min in the 96-well plate. The peptides were further diluted with 2% (v/v) acetonitrile, 0.1 % (v/v) formic acid and between

200 and 300 fmol was loaded on column and injected into the mass spectrometer. SRM assay development was performed with these synthetic peptides on a 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (with NanoSpray® III Source and Emitter Tip Assembly, Applied Biosystems/MDS Sciex) operated with Analyst 1.5 (ABSciex) coupled to an Eksigent Tempo^{TM} nano MDLC (Eksigent, Dublin, CA, USA) LC system equipped with a BioSphere C18 trapping column (1 x 20 mm, 5 µm particles, Nanoseparations, Nieuwkoop, The Netherlands). Peptides were loaded in 0.1 % (v/v) formic acid in water for 5 min at a flow rate of 3 µl/min and then a 60-min linear gradient of 2-35% (v/v) acetonitrile with 0.1 % (v/v) formic acid was developed at a flow rate of 300 nl/min on a Dionex Acclaim PepMap 100 C18 analytical column (0.75 x 150 mm, 3 µm particles, Dionex, Sunnyvale, CA, USA). All SRM transitions for the synthetic peptides were acquired with the following parameters: 2500 V ion spray voltage, a curtain gas setting of 22 p.s.i., a 150 °C interface heater temperature, a declustering potential of 70 and Q1 and Q3 set to unit resolution. In SRM mode a dwell time of 20 ms was used and in the scheduled SRM mode a 2 s cycle time and a 6 min SRM detection window were applied. All transitions generated by TIQAM were tested through SRM with up to 500 transitions per run on pools of the synthetic peptides. MultiQuant 1.2 (Applied Biosystems, Foster City, USA) was used to select five to six transitions with the highest intensity and the best signal-to-noise ratio. The SRM assays for the peptides were selected so that they could be used both on the ABSciex 4000 and 5500 QTRAP according to a multi-instrument peptide fragmentation comparative matrix (U. Kusebauch, *manuscript in preparation*).

Supplementary figure 1



Expression and preliminary characterization of the mastitis QconCAT. Expression of the QconCAT (a) was induced by IPTG and subsequently purified from inclusion bodies by metal affinity chromatography (b). The purified [$^{13}C_6$]arg, [$^{13}C_6$]lys labeled QconCAT was reduced, alkylated and digested with trypsin, and the peptide mass fingerprint was obtained by MALDI-TOF mass spectrometry (c). The specific quantotypic peptides are highlighted on the mass spectrum and in the peptide map at the top of the figure, where the mass of the peptides are given in unlabeled form. Gaps in (a) indicate peptides that could not unambigously be detected in the MALDI-TOF profile.